CHANGES IN THE FAT CONTENT OF THE JAPANESE BEETLE (POPILLIA JAPONICA NEWMAN) DURING METAMORPHOSIS.

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Lipid metabolism has been the subject of much investigation for many years. In the past, studies have been confined chiefly to the vertebrates, although some work has been done on various forms of the invertebrates. Wilber and Bayors (1947) made a study of several marine annelids and reported a wide variation in total lipids and postulated an apparent ratio of these lipids to each other. They indicated a direct relationship between the concentration of cholesterol and that of phospholipids. They stated that cholesterol may be a tissue constituent in the annelids, as reported for the vertebrates by Bloor (1943).

Some work has been done on the fractionation of lipids in insects. Bergmann (1934) reported on the chrysalis oil of the silkworm, *Bombyx mori.* His figures show that 33 per cent of the unsaponifiable fraction is made up of sterols, of which 85 per cent is cholesterol. However, the unsaponifiable fraction is only 1.5 per cent of the total lipids. In the grasshopper, *Melanoplus atlanis,* according to Giral (1946), the free fatty acids make up 74.4 per cent of the total lipids, and the unsaturated predominate over the saturated fatty acids. Finkel, (1948) observed that the fat content for the five day old larvæ of the mealworm, *Tenebrio molitor,* was 7.79 per cent of the wet weight, whereas in 200 day old larvæ, this value increased to 17.4 per cent. The phospholipids for this same period were found to decline from 2.06 to 0.81 per cent of the wet weight. The explanation for this relationship may be, as pointed out by Levenbook (1951), that energy is better stored in the fatty acids of the relatively stable triglyceride than the more soluble phospholipids. Levenbook (1951)

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[1] Submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy at Fordham University.

The author wishes to express sincerest gratitude for the stimulation, interest and critical guidance of Dr. Daniel Ludwig.

observed in the tracheal cells of the horse bot fly *Gastrophilus intestinalis* larva, a great amount of phospholipid at the beginning of the third instar. During the metamorphosis of the blowfly, *Calliphora erythrocephala,* Levenbook (1953) reported no major changes in lipid phosphorus.

The role of lipids in intermediary metabolism during insect metamorphosis has been sadly neglected. Frew (1929) found no change in the fat content during the first part of the pupal stage of the blowfly (species not given) although a decrease was observed in the latter stages. During the life cycle of the tent caterpillar, *Malacosoma americana,* Rudolfs (1926) found that the percentage of ether-soluble materials increased during development, and at an accelerated rate, during the first part of metamorphosis. This increase was followed by a gradual decrease during the latter part of this period. Evans (1932) observed that at 17°C a rapid decrease occurred in the fatty acids of the blowfly, *Lucilia sericata,* up to the eighth day after the larva has stopped feeding, when it decreased more slowly until about the tenth day. At this point he found a definite increase in the fatty acids and later another peak in this synthesis at the fourteenth day. He believed that this latter peak was associated with the onset of histogenesis. Becker (1934) showed a gradual decrease in the fat content during metamorphosis of the mealworm, *Tenebrio molitor,* although the fats of specific organs, which persist into the imago, remained constant. Using the same species, Evans (1934) observed that very little fat is consumed during metamorphosis, but the beginning and end of this period are marked by its utilization. In 1943 Pepper and Hastings, working on the sugar beet webworm, *Loxostege sticticolis,* found no definite changes in the ether-extractable materials although a 40 per cent increase was noted in the saturation of the unsaturated fatty acids. Ludwig and Rothstein (1949), working on the Japanese beetle, *Popillia japonica,* reported a sharp drop in the ether-soluble neutral fat on the fifth and sixth days of pupal life. They also reported that from this point to the emergence of the adult, this fraction of the lipid content gradually decreased in amount.

This review has shown that the information concerning the lipid content of the insects during metamorphosis is very meager. Most of the work has been confined to the four major stages of the life cycle regardless of age or extent of development in the particular stage. Further investigation seems necessary to better understand the role and fate of the lipids during the intermediary metabolism in the insect. In the present investigation, the author has attempted to demonstrate daily changes of some of these lipids during metamorphosis of the Japanese beetle.

MATERIALS AND METHODS

Japanese beetle larvæ were collected in the field from November, 1952, through April, 1953. Second- and third-instar larvæ were brought into the laboratory, each larva being placed in a one-ounce metal salve box containing moistened soil taken from the site of collection. The larvæ were fed wheat, a few grains being added to each box as needed. They were kept in incubators, at a constant temperature of 25°C., until they reached the desired stage of development. In the early months of collection the larvæ were in diapause, a resting stage which usually lasts about 50 days at 25°C. During this period of the life cycle the larvæ feed very little and the boxes were examined weekly. When the diapause was ended, a more frequent check on the larvæ was made due to an increase in activity and feeding.

Observations were made twice each day on the insects in the late pre-pupal stage to obtain the proper age of pupæ. Hence the age of a pupa may be in twelve hour error. When the desired stage of development had been reached, the insects were weighed and killed by placing them individually in small vials containing a mixture of three parts of absolute alcohol and one part absolute ether (Bloor 1943). Aluminum foil was used to cap the vials to assure that no alcohol or ether soluble materials could be removed from the plastic caps used to close the vials. The vials containing the insects were then carefully marked and stored under refrigeration until the time of analysis. The stages in the life cycle chosen for this report included the following: diapause larvæ, postdiapause larvæ, early prepupæ, late prepupæ, newly molted pupæ, pupæ for each day of the ten day pupal period, and newly emerged adults. Separate records were kept on the analysis for male and female adults. In this present investigation, all the determinations made were obtained with the use of the Beckman DU spectrophotometer. Calibration curves were constructed, and from them daily checks could be made both on the instrument used and the technique employed. The fatty acid determinations were made using the method of Bloor (1916a) as modified by Snell and Snell (1937). Measurements of cholesterol were based on the method of Bloor (1916b). The lipid phosphorus determinations were made according to Young-

TABLE 1.

Variations in the Lipids of the Japanese Beetle During Metamorphosis

Values Expressed in Per Cent Wet Weight

Stage of Development	Fatty Acid	Cholesterol	Lipid Phosphorus	Lecithin
Third-instar larva* ..	1.54	......	......	......
Third-instar larva	2.82	0.0372	0.0175	0.4375
Early prepupa	3.29	0.0451	0.0213	0.5325
Late prepupa	3.39	0.0347	0.0250	0.6250
Pupa:				
Just molted	2.67	0.0445	0.0251	0.6275
1-day	2.81	0.0375	0.0269	0.6725
2-day	3.68	0.0552	0.0296	0.7400
3-day	2.18	0.0444	0.0288	0.7200
4-day	3.72	0.0460	0.0288	0.7200
5-day	2.11	0.0408	0.0309	0.7725
6-day	2.67	0.0532	0.0316	0.7900
7-day	3.12	0.0536	0.0293	0.7325
8-day	2.70	0.0424	0.0293	0.7325
9-day	2.77	0.0614	0.0341	0.8525
Adult, just molted:				
Female	2.49	0.0584	0.0481	1.2025
Male	2.01	0.0710	0.0479	1.1975

* Diapause larvæ

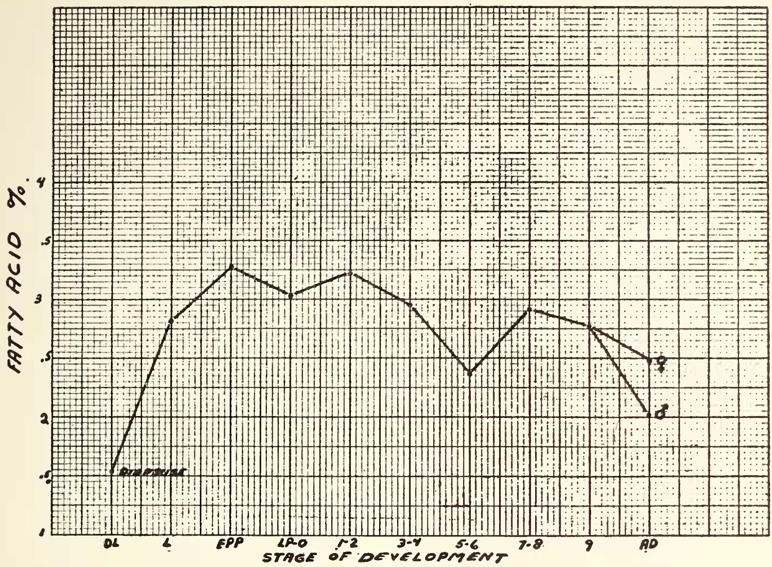

Figure 1. Changes in the fatty acid content during metamorphosis. (Values expressed as per cent of the original wet weight.)

burg and Youngburg (1930). In most cases the above procedures had to be changed slightly to obtain color reactions which would fall in the safe margin of the spectrophotometer scale.

To minimize possible random variations, it was decided to group the averages for successive stages. The groupings used were, diapause larvæ, postdiapause larvæ, early prepupæ, late prepupæ-newly molted pupæ, 1-2 day pupæ, 3-4 day pupæ, 5-6 day pupæ, 7-8 day pupæ, 9 day pupæ, and adults which were recorded separately according to their sex. Since Ludwig and Rothstein (1949) showed a decrease in the free fats during the fifth and sixth days of the pupal stage at 25°C., results of these days were grouped together to determine whether a comparable decrease in the fatty acids occurs at this stage.

RESULTS

The results of the analysis on the fatty acids are given in Figure 1 and Table I. They show that in diapause larvæ the fatty acid content is low, 1.54 per cent of the wet weight. Fatty acids increase to 2.82 per cent during the postdiapause of the larval stage. During the early prepupal stage, there is a sharp increase to 3.29 per cent of the wet weight. Relatively little change is seen in the fatty acid content during the prepupal and early pupal stages, however, a sharp drop occurs during the fifth and sixth days. The value then rises but not to its former level showing a utilization of fatty acids during metamorphosis. The male adult shows a lower fatty acid content than does the female. This difference is probably associated with the storage of lipids in the developing eggs.

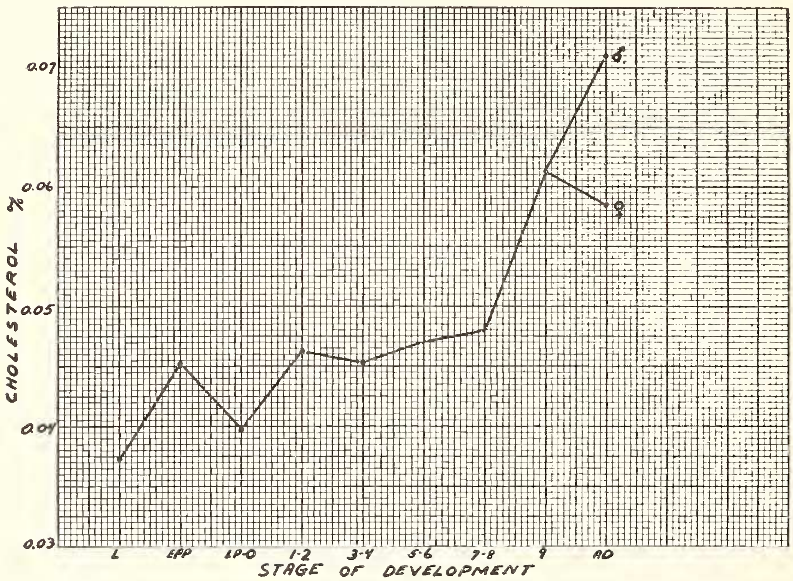

Figure 2. Changes in the cholesterol content during metamorphosis. (Values expressed in per cent of the original wet weight.)

The cholesterol content during metamorphosis is shown in Figure 2 and Table I. In the transition from the larva to the pupa, there is an irregular increase which continues until the eighth day of the pupal period when a very sharp increase is noted during the last day of pupal life. In this fraction the adult male shows a higher concentration than does the female.

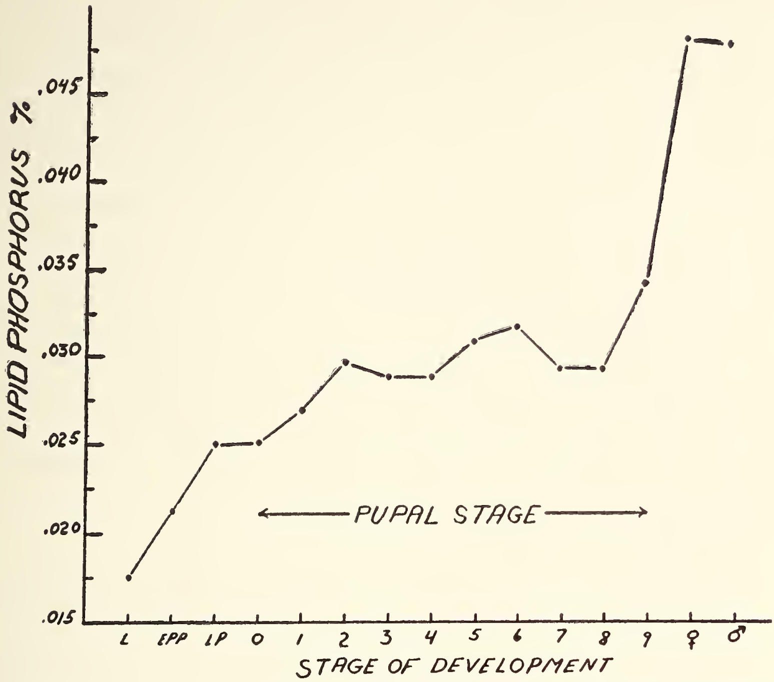

Figure 3. Changes in the lipid phosphorus content during metamorphosis. (Values expressed in per cent of the original wet weight.)

Figure 3 and Table I contain the values obtained for the lipid phosphorus concentrations, which were determined to increase very rapidly during the metamorphosis of the larva to the late prepupa. An irregular but progressive increase is observed during the pupal period. The emergence of the adult is accompanied by a sharp rise in the content of this fraction. The values for the adult male and female are approximately the same.

DISCUSSION

The marked decrease in the fatty acid content observed on the fifth and sixth days of the pupal stage agrees with the work of Ludwig and Rothstein (1949). They, too, found a utilization of fat between the fifth and sixth days. Ludwig and Rothstein (1949) also showed an irregular but rapid decrease in the glycogen content during the first four days of pupal life in the Japanese beetle, an increase on the fifth day, and a gradual decrease throughout the remainder of the pupal period. This decrease during the first four days and increase on the fifth day of pupal life coincides with the reciprocal findings for the fatty acid content in this present investigation. It then becomes evident, from this utilization of glycogen and simultaneous mobilization of the fatty acids during the early days of pupal life, that the main source of energy during this period comes from glycogen and not from fat stores. The increase in glycogen at the fifth day appears to be at the expense of the fatty acids which are observed to drop sharply at this stage, thus indicating a replenishment of the sacrificed glycogen by the fat mobilized. This suggestion agrees with that of Ludwig and Rothstein who believed that the glycogen is formed from lipids. Couvreur (1895) also suggested that, since the increase in glycogen coincides with a decrease in fat, the glycogen may be formed from the fat.

Although there is no apparent synthesis of fatty acid during the last day of the pupal stage, it is possible that the fatty acid formation is still being continued but only in amounts necessary for the vital repair of the adult tissues. That this observation is not limited to the Japanese beetle may be observed in the work of Evans (1932) who worked with the sheep blow fly, *Lucilia sericata.* This investigator found that the total fatty acid content decreased during the early stages of pupation then increased at the time when he believed histogenesis begins. The total fatty acid content then decreased progressively as the organism approached adult life.

The decrease in cholesterol associated with the change from early prepupa to early pupa may be correlated with histolysis which is known to occur at this time (Anderson, 1948). This decrease is followed by a synthesis of cholesterol throughout the pupal period. Since cholesterol is known to be a tissue constituent and not an immediate energy source, it is not inconceivable that this synthesis represents the progressive formation of imaginal tissues. That cholesterol synthesis can occur during periods of mild starvation was shown by Terroine (1914) who worked with canine tissue. Many other investigators obtained similar results working with a variety of other laboratory animals (Bloor, 1943). During the pupal stage of the Japanese beetle, no food is taken in and no waste material is voided except CO_2 and possibly water, hence this stage in the life cycle can be considered a period of starvation. Although fluctuations in the cholesterol content were observed, the general trend is upward during metamorphosis.

The results obtained in the lipid phosphorus studies show a progressive rise in this fraction during metamorphosis. It can be noted that during the larval stage, which is a period of feeding, the lipid phosphorus content shows a marked increase indicating that the fat stores are being built up. This fact is further strengthened by the observation that the fatty acids and free fats are also being stored at this time. During the relatively inactive pupal period, there is an increase in the lipid phosphorus content but at a less rapid rate. This slower increase may be due to the fact that food intake has ceased thereby decreasing the amount of fat being transported to the depots. It is of interest to note that the lipid phosphorus content is increased moderately at about the fifth and sixth days of pupal life. This increase may be correlated with a simultaneous decrease in the fatty acid content, indicating that part of the available fatty acids are being incorporated in the formation of phospholipids which act as carriers of fats to and from the depots. In this particular instance the increase in the phospholipids may be indicative of fat utilization, supplying energy directly for the histogenesis of imaginal tissues, or indirectly, through the synthesis of glycogen, which is known to increase at this time. This consideration is further substantiated by the fact that toward the end of the pupal stage, an additional increase in the lipid phosphorus content occurs, thus indicating that fat stores are being utilized to provide energy needed for the development and differentiation of new adult tissues.

SUMMARY

Determinations were made on the fatty acid, cholesterol, and lipid phosphorus content of the Japanese beetles at various stages of the life cycle.

The fatty acid content was seen to increase during the first stages of metamorphosis, decrease sharply on the fifth and sixth days of pupal life and then gradually build up on the seventh day of pupal life and to diminish slightly toward the emergence of the adult.

The cholesterol content was seen to increase gradually during the entire life cycle. The synthesis was distinct and progressive during the pupal period.

An increase in the lipid phosphorus content was evident during metamorphosis. It is known that phospholipids act as carriers to and from the depots and hence an increase in the phospholipids is indicative of fat mobilization or utilization during metamorphosis.

LITERATURE CITED

ANDERSON, J. M. 1948. Changes in the distribution of nitrogen in the Japanese beetle (*Popillia japonica* Newman) during metamorphosis. Physiol. Zool., 21: 237-252.

BECKER, M. 1934. Wandlungen des Fettes während der Metamorphose. Biochem. Zeitsch. 272: 227-234.

BERGMANN, W. 1934. Fats and sterols in *Bombyx mori*. Jour. of Biol. Chem. 107: 527-532.

BLOOR, W. R. 1916a. The determination of cholesterol in blood. Jour. of Biol. Chem. 24: 227-231.

———. 1916b. The distribution of the lipids (fat) in human blood. Jour. of Biol. Chem. 25: 577-599.

———. 1943. Biochemistry of the fatty acids and their compounds, the lipids. Reinhold. New York.

COUVREUR, E. 1895. Sur la transformation de la graisse en glycogène chez le ver à soie pendant la métamorphose. Compt. rend. de la Soc. biol. 47: 796-798.

EVANS, A. C. 1932. Some aspects of chemical changes during insect metamorphosis. Jour. of Exper. Biol. 9: 314-322.

———. 1934. On the chemical changes associated with metamorphosis in a beetle, *Tenebrio molitor* L. Jour. of Exper. Biol. 11: 397-401.

FINKEL, A. J. 1948. The lipid composition of *Tenebrio molitor* larvæ. Physiol. Zoöl. 21: 111-133.

FREW, J. G. H. 1929. Studies in the metabolism of insect metamorphosis, Brit. Jour. of Exper. Biol. 6: 205-218.

GIRAL, F. 1946. Fats of insects. V. *Sphenarium purpurascens* Charpentier, Jour. of Biol. Chem. 162: 61-63.

LUDWIG, D., AND F. ROTHSTEIN. 1949. Changes in the carbohydrate and fat content of the Japanese beetle, *Popillia japonica* Newman, during metamorphosis. Physiol. Zoöl. 22: 308-317.

LEVENBOOK, L. 1951. The variation in fat and glycogen content of the bot fly, *Gastrophilus intestinalis,* larva tracheal organ during development. Jour. of Exper. Biol. 28: 173-180.

———. 1953. The variation in phosphorus compounds during metamorphosis of the blow fly, *Calliphora erythrocephala* Meig. Jour. of Cellular and Comp. Physiol. 41: 313-334.

PEPPER, J. H., AND E. HASTINGS. 1943. Biochemical studies on the sugar beet web-worm, *Loxostege sticticolis* L., with special reference to the fatty acids and their relation to diapause and sterility. Mont. Agr. Exp. Sta. Bull. 413: 1-36.

RUDOLFS, W. 1926. Studies on chemical changes during the life cycle of the tent caterpillar. Jour. of the New York Ent. Soc. 40: 481-488.

SNELL, F. D. AND C. T. SNELL. 1937. Colorimetric methods of analysis. Van Nostrand. New York.

TERROINE, E. F. 1914. Nouvelles recherches sur l'influence de l'inanition et de la suralimentation sur la teneur des tissus en substances grasses et en cholestérine. Jour. de Physiol. et de Path. gen. 16: 408-418.

WILBER, C. G., AND W. M. BAYORS. 1947. A comparative study of the lipids in some marine annelids. Biol. Bull. 93: 99-101.

YOUNGBURG, G. E., AND M. V. YOUNGBURG. 1930. A system of blood phosphorus analysis. Jour. of Lab. and Clin. Med. 16: 159-166.